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PERSPECTIVES IN RENAL MEDICINE

Chemokines and chemokine receptors are involved in the resolution or progression of renal disease

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Chemokines and chemokine receptors are involved in the resolution or progression of renal disease. Locally secreted chemokines mediate leukocyte recruitment during the initiation and amplification phase of renal inflammation. In turn, the infiltrating leukocytes contribute to renal damage by releasing inflammatory and profibrotic factors. Rapid down modulation of the chemokine signal will support resolution of acute inflammation, whereas progression occurs if ongoing or repeated renal injury maintains continuous local chemokine secretion and leukocyte influx into the glomerulus or the interstitial space. In glomerular injury proteinuria itself as well as glomerular secreted cytokines stimulate downstream tubular epithelial cells to also secrete chemokines. During primary tubular injury, tubular epithelial cells directly become a major site of chemokine production. This in turn supports leukocyte infiltration and activation. Infiltrating leukocytes stimulate fibroblast proliferation and matrix synthesis, leading to widening of the interstitial space. The specific and intricate renal vascular architecture renders the organ susceptible to ischemic damage as interstitial volume increases. Ischemia in turn serves as a stimulus for chemokine and cytokine production and matrix synthesis. The mutual stimulation between fibroblasts and infiltrating leukocytes supports progressive tubular damage, renal fibrosis, and glomerulosclerosis. Potentially this vicious circle leading to progression of chronic nephropathies offers the opportunity for therapeutic intervention. Interfering with the chemokine network that mediates leukocyte recruitment may represent a promising therapeutic option for progressive renal disorders and renal fibrosis. This article summarizes the present data on the role of chemokines in acute and chronic renal disease with special emphasis on their potential role in mediating resolution or progression of renal disease as well as on therapeutic options.

Progression of the various nephropathies to end-stage renal disease remains a major problem in nephrology as only some renal diseases resolve after an acute phase whereas most tend to become chronic. The resulting slow but continuous decline of renal function is the most common cause of end-stage renal failure, and this process correlates with progressive tubulointerstitial injury and renal fibrosis [1–4]. Progressive interstitial fibrosis is characterized by the accumulation of leukocytes, fibroblasts, extracellular fibrous matrix as well as tubular atrophy [5]. In this process the accumulation of interstitial leukocytes is critical for mediating fibroblast proliferation, differentiation into myofibroblasts, matrix production, and tubular damage as infiltrating macrophages and lymphocytes are major sources for proinflammatory and profibrotic cytokines [6]. Therefore, understanding the mechanisms that direct circulating leukocytes to and maintain them in the interstitial space may offer new therapeutic targets of progressive renal disease. In this context, the concerted interaction of chemokines and adhesion molecules have been shown to play important roles not only in the control of leukocyte recruitment, activation, and effector function, but also in the modulation of angiogenesis and aspects of adaptive immunity [7–12]. As these diverse biologic processes are all involved in progressive renal disease and fibrosis, the chemokine system is a potential target for therapeutic intervention. Previous reviews centered mostly on the role of chemokines in acute glomerular and interstitial inflammation [reviewed in 8]. In view of the hypothesis that blockade of leukocyte infiltration may be a valuable approach for the treatment of progressive renal disease, our current review focuses on the biology of chemokines and chemokine receptors in the context of resolution or progression of renal disorders. First, a model of the role of chemokines in the various stages of renal disease is presented. In the second part, the regulation of resolution or progression and possible therapeutic implications of chemokine blockade are discussed.

Key words: tubular damage, renal fibrosis, glomerulosclerosis, leukocyte recruitment, end-stage renal failure, profibrotic cytokines.

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MODEL OF STAGES OF PROGRESSIVE RENAL DISORDERS

The course of progressive renal disorders can be arbitrarily divided into four phases: the initiation phase, the amplification phase, the progression phase, and the terminal phase (Fig. 1).

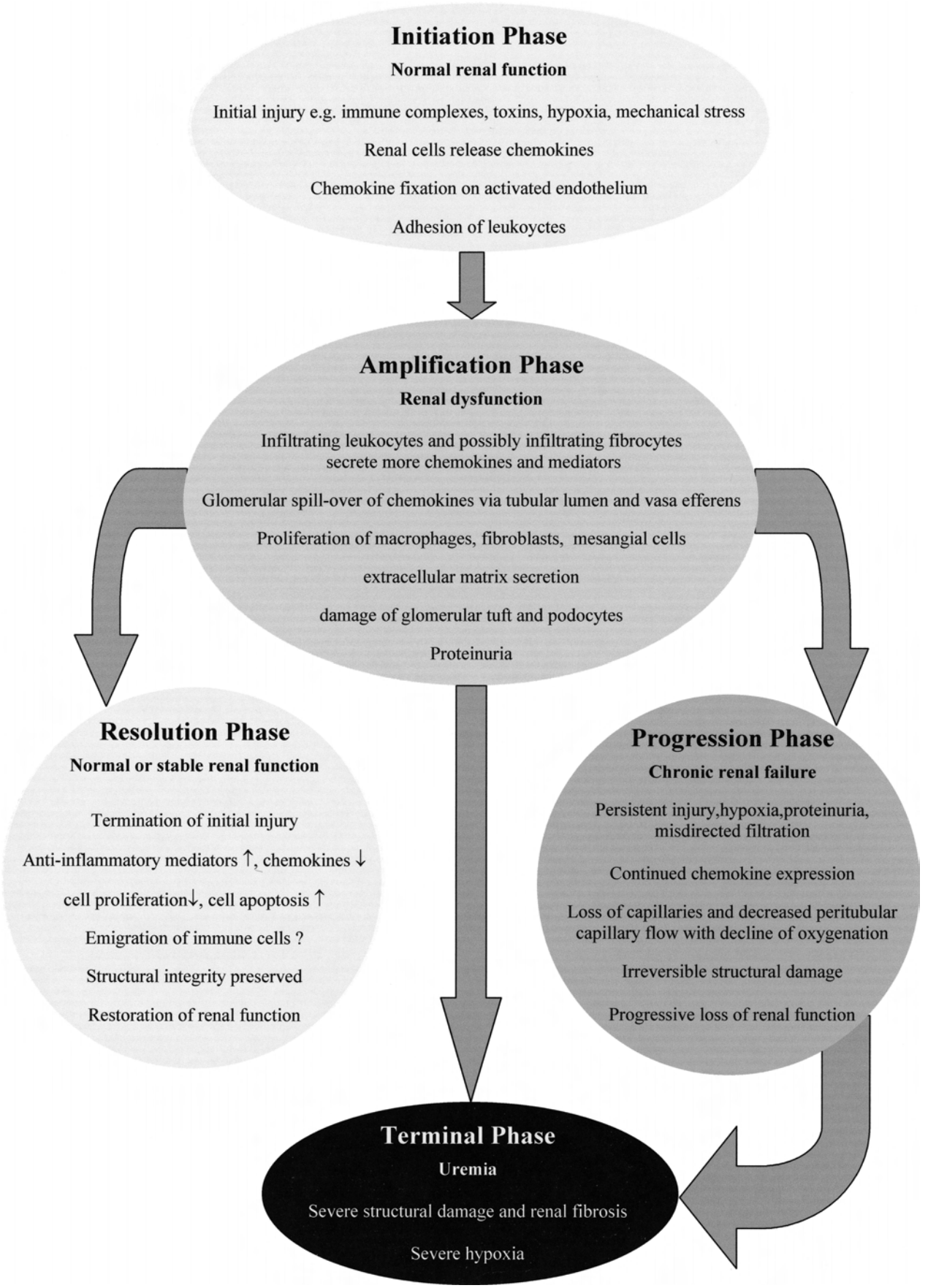

Fig. 1. Proposed model of chemokine involvement in progressive renal disease and fibrosis. Details are in the text.

Initiation phase

Injury to any type of renal parenchymal cells leads to the secretion of proinflammatory mediators that induce leukocyte infiltration and activation at the place of injury (Fig. 2A). If the inflammatory process is restricted either to the glomerulus or to the tubulointerstitium the leukocyte infiltration will be restricted to the respective compartment [13, 14]. The selective recruitment of certain leukocyte subsets to different compartments of the kidney further illustrates the complexity of this process. For example, except for transplant glomerulitis, T cells are rarely found within the glomerular tuft as long as Bowman's capsule is intact, whereas T cells are commonly present in interstitial infiltrates [15]. Compared to peritubular vessels glomerular capillaries may not support the binding and transmigration of T cells, a phenomenon that could be related to a different expression of adhesion molecules and chemokines or simply to higher shear stress in the glomerular microcirculation. On the other hand, macrophages can be found intraglomerularly during proliferative and especially crescentic glomerulonephritis. Microthrombosis of glomerular capillaries, which is commonly present in focal necrotic or crescentic lesions, may contribute to this phenomenon.

Amplification phase

Infiltration and local proliferation of leukocytes further enhance the local production of cytokines and chemokines (Fig. 2B). Furthermore, neutrophils and macrophages generate radical oxygen species and lipid mediators that contribute to local tissue damage, supporting positive feedback mechanisms. Macrophages themselves may secrete extracellular matrix components, but they also are the major source of growth factors such as fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), epithelial growth factor (EGF), and platelet-derived growth factor (PDGF) [6]. These cytokines stimulate mesangial cell proliferation and matrix synthesis in the glomerulus leading to the typical pictures of mesangioproliferative glomerulonephritis (GN) [16]. Activation of podocytes leads to rearrangement of the complex secondary structure, including the slit membrane leading to foot process effacement and proteinuria. Extensive podocyte damage leads to focal adhesions of the denuded GBM to Bowman's capsule and to focal glomerulosclerosis.

In the tubulointerstitium, fibroblast proliferation and secretion of extracellular matrix leads to widening of the interstitial space and renal fibrogenesis. Sources of the heterogeneous fibroblast population include proliferation of resident fibroblasts and myofibroblasts derived from tubular epithelial cells by a process described as epithelial-mesenchymal transformation, two mechanisms that are induced by macrophage derived profibrotic cytokines such as FGF-2 [17]. In addition, blood borne immature, monocyte-like cells, referred to as fibrocytes, rapidly enter sites of tissue injury and contribute to the local fibrosis [18]. However, their role in renal fibrosis has not yet been determined.

Another common observation leading to interstitial fibrosis is the appearance of an interstitial cell infiltrate in primary glomerulopathies such as membranous GN, focal glomerulosclerosis or mesangioproliferative GN. The tubular epithelial cell may have an important role in mediating the progression from glomerular to tubulointerstitial nephritis. Spillover of proinflammatory mediators, growth factors, and even albumin into the glomerular ultrafiltrate appear as stimulators for tubular epithelial cells to secrete additional proinflammatory profibrotic cytokines and chemokines [8]. Furthermore, proinflammatory mediators secreted within the glomerulus will reach the post-glomerular peritubular circulation, thereby activating peritubular endothelial and tubular epithelial cells [19]. In addition, focal capsular adhesions develop "misdirected" ultrafiltrate that may result in local generation of mediators [20]. All of these mechanisms may enhance interstitial mononuclear cell recruitment secondary to primary glomerular injury and thus expand the lesion from the glomerulus to the tubulointerstitium.

Progression phase

The continuous stimulation of intrinsic renal parenchymal cells by infiltrating leukocytes, proteinuria, and secreted cytokines results in ongoing synthesis of extracellular matrix components and irreversible structural damage (Fig. 2C). In the glomerulus infiltrating macrophages stimulate mesangial cells to secrete collagen type IV, laminin, and fibronectin that contribute to the development of glomerulosclerosis [21]. Mesangial expansion also leads to narrowing or obliteration of single glomerular capillaries as well as dilation of others [22]. Eventually this will not only result in podocyte damage and glomerular sclerosis, but also in destruction of the entire nephron, including downstream peritubular capillaries [20, 22]. Thus, the tubulointerstitial compartment undergoes major structural rearrangement. The accumulation of T cells and macrophages provides continuous release of profibrotic mediators that induce the accumulation of fibroblasts, and the ongoing production of extracellular matrix. Activated tubular epithelial cells themselves contribute to this phenomenon by matrix production chemokine-cytokine release, and even to *trans*differentiate to myofibroblasts that migrate into the interstitial space [17]. The interstitial cell infiltrate itself, together with the increasing amount of extracellular matrix, lead to critical widening of the interstitial space, thereby increasing the distance of the remaining peritubular capillaries to their respective tubular segments, impairing oxygen diffusion as well as tubular reabsorption and excretory function

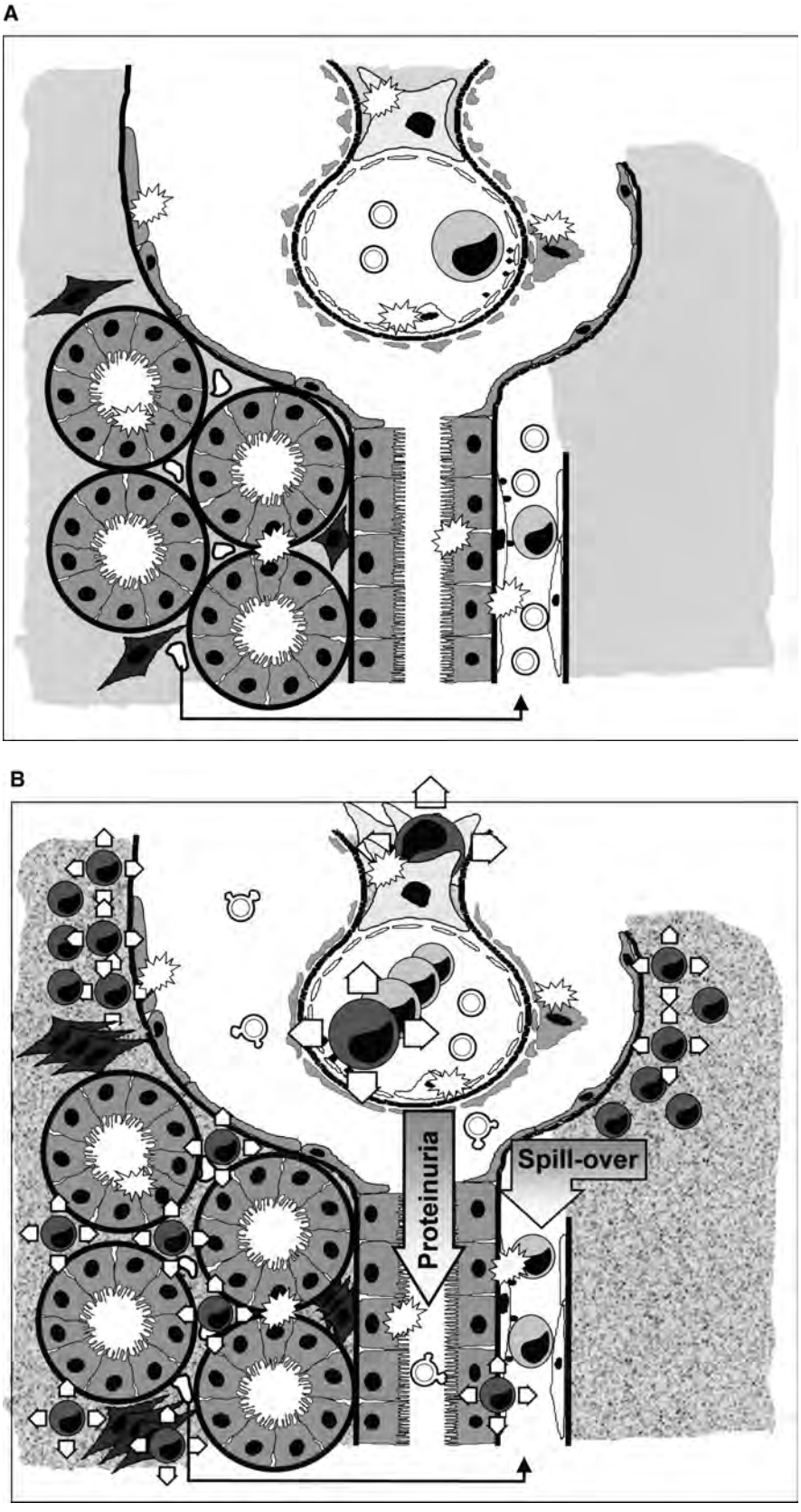

Fig. 2. (*A*) Initiation phase. All types of intrinsic renal cells can secrete chemokines as a response to immunologic, toxic, ischemic or mechanical injury (✲). The selective expression of adhesion molecules and chemokines on endothelial cells of glomerular or peritubular capillaries supports leukocyte arrest and transmigration either into the mesangium or the interstitial space. (*B*) Amplification phase. As long as the initial stimulus persists, infiltrating leukocytes release lipid mediators, cytokines, and chemokines, leading to glomerular and/or tubulointerstitial inflammation, which results in hematuria, leukocyturia, and proteinuria. Proinflammatory and profibrotic cytokines stimulate the proliferation of mesangial cells in the glomerulus and of interstitial fibroblasts in the interstitium. Upon stimulation activated mesangial cells and interstitial fibroblasts increase the synthesis of extracellular matrix components. Leukocyte infiltration, fibroblast proliferation, matrix deposition as well as edema will increase the interstitial volume.

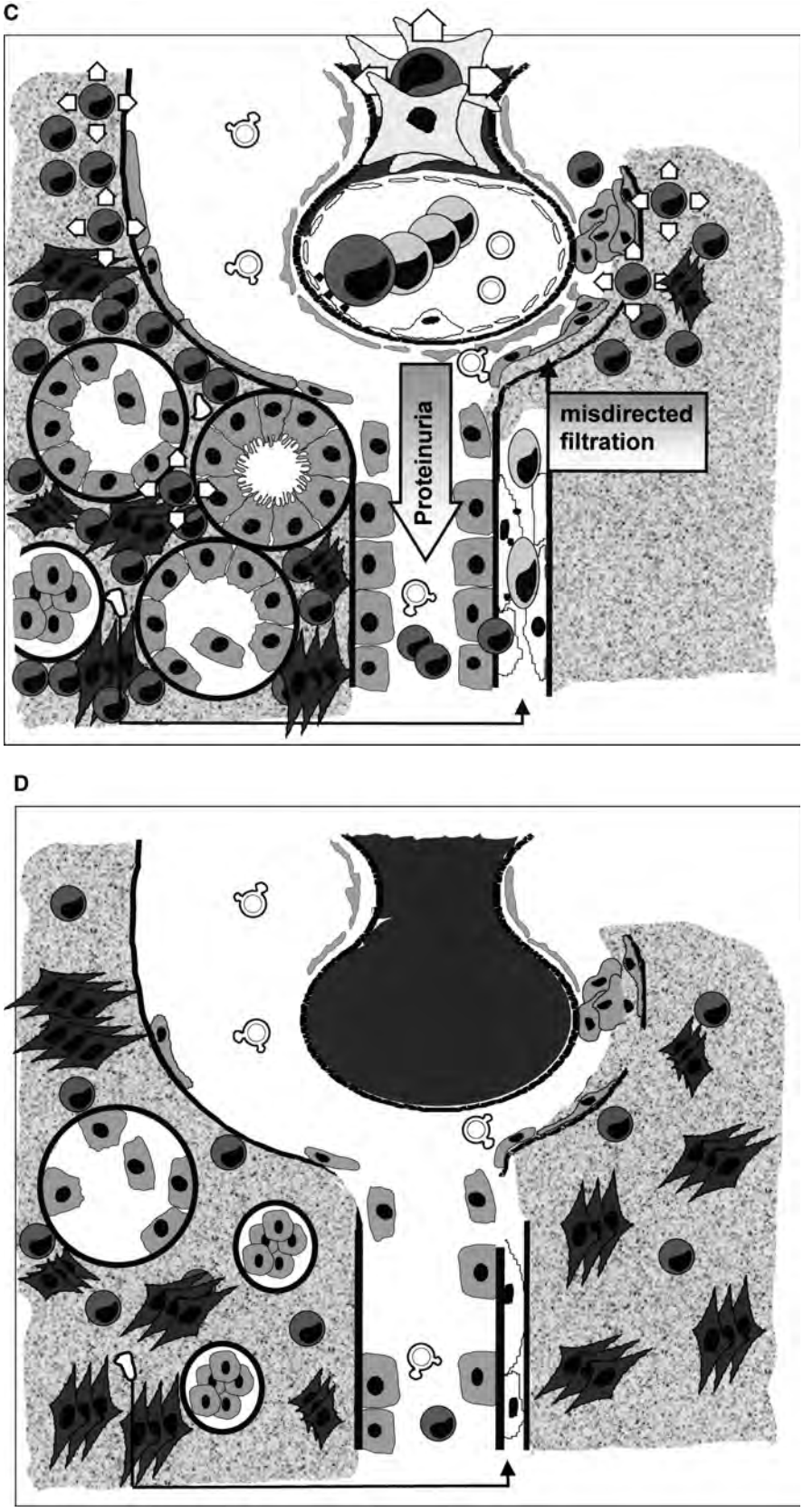

Fig. 2. (Continued) (*C*) Progression phase. Although the initial stimulus may have already subsided, the structural damage of acute inflammation may have become irreversible. Focal glomerulosclerosis with misdirected filtration and proteinuria maintains a persistent signal for tubular epithelial cells to release chemokines into the interstitium. Overspill of locally secreted cytokines and chemokines also supports downstream inflammation of the renal interstitium. Massive increase of the interstitial volume and destruction of peritubular capillaries supports renal ischemia leading to tubular atrophy, secondary glomerulosclerosis, and progressive renal dysfunction. (*D*) Terminal phase. Continuous destruction of the peritubular capillary network and tubular segments further support renal ischemia, which is a strong stimulus for fibroblast proliferation and matrix synthesis via autocrine mechanisms. Myofibroblasts support tissue contraction up to the ultimate stage, the end-stage shrunken kidney.

Table 1. Chemokines and chemokine receptors

Ligand		Receptors	Function	Ligand		Receptors	Function
CCL1	I-309	CCR8	i	CXCL1	Groα	CXCR1,2	i
CCL2	MCP-1	CCR2	i	CXCL2	Groβ		i
CCL3	MIP-1α	CCR1,5	i	CXCL3	Groγ		i
CCL4	MIP-1β	CCR5	i	CXCL4	PF4	CXCR1,2	i
CCL5	RANTES	CCR1,3,5	i	CXCL5	ENA-78	CXCR2	i
CCL7	MCP-3	CCR1,2	i	CXCL6	GCP-2	CXCR1,2	i
CCL8	MCP-2	CCR1,2,5	i	CXCL7	NAP-2	CXCR2	i
CCL11	Eotaxin	CCR3	i	CXCL8	IL-8	CXCR1,2	i
CCL13	MCP-4	CCR1,2,3	i	CXCL9	Mig	CXCR3	i
CCL14	HCC-1	CCR1	i	CXCL10	IP-10	CXCR3	i
CCL15	HCC-2	CCR1	i	CXCL11	I-TAC	CXCR3	
CCL16	HCC-4	CCR1,8	i	CXCL12	SDF-1	CXCR4	h
CCL17	TARC	CCR4	i	CXCL13	BCA-1	CXCR5	
CCL18	DC-CK1			CXCL14	Bolekine		
CCL19	ELC	CCR7	h	CXCL15	Lungkine		h
CCL20	LARC	CCR6					
CCL21	SLC/6Ckine	CCR7	h	XCL			
CCL22	MDC	CCR4	i	XCL1	lymphotactin	XCR1	i
CCL23	MPIF-1	CCR1		XCL2	SCM-1β		i
CCL24	Eotaxin-2	CCR3	i				
CCL25	TECK	CCR9	i				
CCL26	Eotaxin-3	CCR3	i	CX_3CL			
CCL27	CTAK/Eskine	CCR10		CX_3CL1	Fractalkine	CX_3CR1	h,i

Abbreviations are: h, homeostatic, i, inflammatory.

[23]. The tubulointerstitial ischemia is considered to be an important factor for tubular cell apoptosis, necrosis, and, finally, tubular atrophy [24, 25]. Thus, progressive glomerular and interstitial injuries are tightly linked and aggravate each other by multiple mechanisms, including ischemia.

Terminal phase

Finally, vascular rarification and diffuse scarring lead to extensive tubular atrophy, and glomerulosclerosis (Fig. 2D). The extensive loss of renal parenchyma and structural integrity finally results in end-stage renal disease with the clinical signs and symptoms of uremia. Leukocytic cell infiltrates resolve, but renal fibroblasts maintain the synthesis of extracellular matrix due to sustained hypoxia and autocrine stimulation [26, 27]. Myofibroblasts contribute to contraction of the fibrous tissue with scarring, resulting in the ultimate stage, the shrunken kidney.

In the above process there are roles for chemokines at multiple steps. The contribution of the chemokines cannot be viewed in isolation, but as part of an integral system together with adhesion molecules and cytokines.

STRUCTURAL AND FUNCTIONAL CLASSIFICATION OF CHEMOKINES AND CHEMOKINE RECEPTORS

Chemokines are low molecular weight cytokines that were first characterized by their ability to induce directed migration of leukocytes [28]. To date, more than 44 chemokines and 21 chemokine receptors have been described (Table 1). The chemokine superfamily can be divided into four branches (C, CC, CXC, and CX_3C), based upon the position of the first two cysteine residues in a four-cysteine motif in their primary amino acid sequence [reviewed in 29]. These cysteines can be separated by one or three additional amino acids (designated as X). The official nomenclature used to describe individual chemokines is based upon the class it belongs to, for example, CC, CXC, CX_3C, and C chemokines [28]. A subgroup of CXC chemokines displays the additional amino acid motif E-L-R-CXC (glutamic acid-leucine-arginine-cysteine-X-cysteine). The E-L-R-CXC chemokines generally act as neutrophil chemoattractants and promotors of angiogenesis, while the CXC chemokines without the E-L-R motif bind to different receptors, are more active on lymphocytes, and inhibit angiogenesis. Lymphotactin-α and -β are the only C chemokines described. They share homology with CC chemokines at their carboxyl end, but lack the first and third cysteines in the four-cysteine motif [30]. Fractalkine, the only known CX_3C chemokine, has three additional amino acids between the first two cysteine residues. It is tethered directly to the cell membrane via a mucin stalk and combines the function of a chemokine and adhesion molecule [31]. By the new systematic nomenclature, chemokines are classified by the above criteria and their binding characteristics as ligands (L), such as, CL, CCL, CXCL and CX_3CL.

Chemokines can be further classified according to function and regulation of expression as inflammatory or homeostatic [10, 31]. The inflammatory chemokines

are up-regulated by proinflammatory stimuli and orchestrate innate and adaptive immune responses, such as regulators of T cell differentiation [32]. The homeostatic group of chemokines modulates lymphocyte and dendritic cell trafficking during immune surveillance [33]. All members of the chemokine family work in concert with selectins and integrins to sort and direct effector leukocyte migration [29, 34]. In addition chemokines have been shown to play important roles in the control of leukocyte activation and effector function.

The biologic action of chemokines is mediated through a large family of seven-transmembrane-spanning G protein-coupled receptors [7, 10, 12]. Chemokine receptors are designated according to the class of the chemokine ligands they bind, for example, CR, CCR, CXCR, and CX_3CR. Each chemokine receptor has a distinct chemokine specificity and a restricted expression on subclasses of leukocytes and non-hematopoietic cells. However, the ligand specificities of the receptors can substantially overlap within a chemokine class leading to a high degree of redundancy. Some receptors bind multiple chemokines, and some chemokine ligands bind to multiple receptors [reviewed in 29, 31]. In general, the proinflammatory chemokine receptors have more promiscuous ligand binding specificities, while receptors involved in normal leukocyte trafficking have fewer ligands. In addition, it has been proposed that some chemokine receptors form homo- or heterodimers that may alter their function or sensitivity to chemokine stimulation [reviewed in 29, 31].

CHEMOKINES MEDIATE LEUKOCYTE TRAFFICKING AT MULTIPLE STAGES

Chemokines mediate events at multiple stages in the process of leukocyte-endothelium interaction and transmigration [8, 31]. Injured renal cells produce chemokines and many other inflammatory cytokines. This in turn enhances the expression of adhesion molecules on endothelial cells. As an early event reversible rolling of leukocytes along the endothelial surface occurs through transient interactions between selectins and vascular addressins. Rolling leukocytes are brought into contact with chemokines, which are retained on heparan sulfate proteoglycans of the endothelial surface following secretion by, for example, activated endothelial cells or subendothelial renal cells. The chemokines bind to the respective chemokine receptors of the rolling leukocytes and thereby activate leukocyte-expressed integrins, resulting in shear-resistant firm adhesion of the leukocyte to the endothelial surface as a prerequisite for leukocyte emigration [31, 34]. Specific but apparently redundant chemokines appear to differentially influence spreading, diapedesis, and subsequent migration into the tissue space [31, 35]. For example, monocytes and T helper-1 (Th1)-like T cells express both chemokine receptors CCR1 and CCR5, which share the ligand CCL5/RANTES [36]. In an in vitro system it was found that immobilized CCL5/RANTES (regulated upon activation, normal T cell expressed and secreted) induced leukocyte arrest via CCR1, while leukocyte spreading was mediated via CCR5, and *trans*-endothelial migration was supported by both receptors [36]. In this context it is of interest that chemokines also modulate the redistribution of junctional adhesion molecules (JAMs) of endothelial cell tight junctions that may promote leukocyte diapedesis by transient opening of focal cell-cell contacts [37]. Furthermore, chemokines such as CCL5/RANTES up-regulate the secretion and activity of matrix metalloproteinases by infiltrating leukocytes [38], thus facilitating leukocyte transmigration through the basement membrane and extracellular matrix [39]. Chemokines such as CXCL8/IL8 also activate infiltrating leukocytes inducing granule release and respiratory burst [40, 41]. Finally, CCL5/RANTES can function as a co-stimulatory agent in T cell proliferation [42], so that chemokines are involved in multiple steps from adhesion, transmigration, and activation to local proliferation of leukocytes at the site of tissue injury.

CHEMOKINES MEDIATE RENAL INFLAMMATION DURING THE INITIATION AND AMPLIFICATION PHASE

In vitro studies

All types of renal cells (that is, endothelial, mesangial, tubular epithelial, interstitial cells, and podocytes) can express chemokines upon stimulation in vitro [reviewed in 8]. In general, proinflammatory stimuli such as TNF-α, interleukin-1β (IL-1β) interferon-γ (IFN-γ), and lipopolysaccharide (LPS) rapidly induce CCL2/MCP-1, CXL8/IL-8, and CXCL10/IP-10 within few hours. CCL5/RANTES is induced in a more delayed manner after 12 to 48 hours [8]. Reactive oxygen species generation may represent a common mechanism of injury-induced chemokine generation [43]. Growth factors and vasoactive agents like angiotensin II also may stimulate chemokine production of certain cell types (for example, endothelial cells) [44]. Furthermore, immune complexes and complement activation cause mesangial production of chemokines [8], and in proximal tubular cells chemokines can be induced by high concentrations of albumin [45, 46].

Descriptive in vivo studies

Several studies have characterized the expression patterns of the chemokines in animal models of acute glomerular or tubulointerstitial disease. They demonstrated that various inflammatory chemokines such as CCL2/monocyte chemoattractant protein-1 (MCP-1), CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES are expressed only in the diseased compartment of the kidney [reviewed

in 8]. Renal chemokine expression has been found to correlate with the local accumulation of leukocytic effector cells and renal damage. The Duffy antigen receptor for chemokines (DARC) binds multiple chemokines including CCL5/RANTES, but lacks signaling function. In the normal kidney DARC is only and is expressed on post-capillary venules [47]. During various forms of renal diseases DARC expression expands to most peritubular vessel endothelium [47]. DARC might serve as an endothelial presentation molecule for chemokines involved in signaling of specific exit sites for chemokine positive cells into the interstitium.

The relevance of the animal data for human disease has been confirmed by a variety of human biopsy studies that have analyzed the chemokine expression patterns in renal diseases [reviewed in 8]. Local expression of chemokines also may contribute to another common observation made during active GN, that is, the periglomerular accumulation of macrophages and T cells. Immunohistological and in situ hybridization studies have demonstrated chemokine expression by parietal glomerular epithelial cells [48–50]. The release of chemokines by these cells into the surrounding interstitium may facilitate the periglomerular accumulation of leukocytes.

Besides intrinsic renal cells, infiltrating leukocytes become a major source of local chemokine production in a positive amplification loop [13, 14]. Chemokines secreted during the initiation phase of injury also may target intrinsic renal cells. The local production of CXCL10/IP-10 could induce proliferation of mesangial cells presumably via the CXCR3 receptor [51]. CCR7, the receptor for CCL19/ELC and CCL21/SLC is expressed on mesangial cells and mediates proliferative and anti-apoptotic effects [52]. The functional role of CCR1 expression by mesangial cells upon stimulation by CCL5/RANTES remains to be elucidated [53].

Functional studies in animal models of renal diseases

The role of chemokines in the pathogenesis of renal inflammation was examined by blocking chemokine activity with neutralizing antibodies, chemokine receptor antagonists, and targeted disruption of genes encoding chemokines and their receptors in various animal models [8, 54]. These studies yield in part conflicting results concerning the role of chemokines illustrating the multifaceted role of these mediators. For example, neutralizing antibodies against CCL2/MCP-1 reduced infiltration of glomerular macrophages in rat anti-Thy-1.1 nephritis [55]. Treatment with anti-CCL2/MCP-1 antibodies reduced proteinuria and monocyte influx in rat nephrotoxic serum nephritis [56, 57] and abrogated crescent formation and leukocyte infiltration in murine nephrotoxic nephritis [58]. However, in CCL2/MCP-1 deficient mice given nephrotoxic serum the glomerular injury persisted while the tubulointerstitial injury was reduced [59]. In this model tubular CCL2/MCP-1 expression was more prominent than glomerular expression, which may explain the localized effect [59]. A similar reduction of tubulointerstitial damage was obtained with a CCL2/MCP-1 antisense approach in rats with nephrotoxic serum nephritis [60]. A role of CX_3CL1/fractalkine in mediating renal leukocyte infiltration and inflammatory injury was demonstrated recently. Treatment with a neutralizing antibody against the CX_3CL1/fractalkine receptor CX_3CR1 improved renal function and prevented crescentic glomerulonephritis in the rat nephrotoxic serum nephritis model [61]. Using the CCL5/RANTES antagonist Met-RANTES Lloyd et al showed in nephrotoxic serum nephritis that mice treated with Met-RANTES had reduced proteinuria, T cell, and macrophage infiltration [58]. The CCL5/RANTES antagonist AOP-RANTES inhibited glomerular macrophage infiltration and collagen IV deposition in the anti-Thy-1.1 model [62]. A virus-derived antagonist blocking multiple chemokine receptors, vMIP-2, decreased proteinuria and interstitial leukocyte infiltration in rat nephrotoxic serum nephritis [63]. These rat and mouse studies in different models of glomerular injury would support roles for CCL5/RANTES and CCL2/MCP-1 in the initiation of the leukocyte influx and the resulting proteinuria (abstract; Pi et al, *J Am Soc Nephrol* 12:A4608, 2001). Surprisingly, however, studies with mice with deletions of the genes encoding for these chemokines or their receptors have yielded partially conflicting results [59, 64–66]. For example, mice with targeted gene deletion of CCL2/MCP-1 had reduced interstitial leukocyte infiltration and tubular injury during the nephrotoxic serum nephritis [59]. However, glomerular histopathology was not affected, which may relate to the particular glomerular lesions of the nephrotoxic serum model in mice that lack major glomerular macrophage infiltration [60, 67]. However, another group found a marked reduction of crescent formation with a CCL2/MCP-1 blocking antibody in this model [58]. In mice lacking the CCR2 receptor for CCL2/MCP-1 renal pathology after nephrotoxic serum was worse despite reduced glomerular macrophage infiltration, indicating that lack of CCR2 may influence other immune mechanisms besides the local cell infiltration [64]. Conflicting results also were obtained with chemokine receptor antagonists and knockout mice for the chemokine receptors CCR1 and CXCR3 with this model [58, 61, 66]. Although interpretation of data derived from the nephrotoxic serum nephritis model is often difficult due to different preparations of nephrotoxic antisera and the different mouse strains used [67–69], the reasons for the discrepant results with antagonist, blocking antibodies, and knockout mice remain unclear. In the case of mutant mice they could involve the development of compensatory chemokine and receptor systems or alterations in the immune response. The latter could occur also with the use of chemokine

antagonists and blocking antibodies. Potential immune modulation secondary manipulating the chemokine system is discussed later in this review.

In summary, a wealth of data demonstrates the expression of chemokines in renal inflammation and tissue damage. Despite considerable in vitro redundancy of chemokine-chemokine receptor interaction, in vivo specific chemokines can mediate unique and relevant effects. However, at present conflicting results with chemokine antagonists and chemokine receptor knockout mice in models of acute renal inflammation do not allow a simplistic antichemotactic approach to the therapy of acute inflammatory renal disorders [54]. More in depth studies on potential systemic immunmodulatory effects of disruption of specific chemokines and their receptors will be required prior to the design of therapeutic trials with chemokine antagonists.

CHEMOKINES MEDIATE INFLAMMATION DURING THE PROGRESSION OF RENAL DISEASES

In vitro studies

Tubulointerstitial leukocyte recruitment and activation depends on chemokine-mediated processes and interstitial fibroblasts will produce chemokines during hypoxia, hyperglycemia and upon stimulation with proinflammatory cytokines [8, 70–73]. In turn, chemokines may directly mediate profibrogenic effects through receptors on parenchymal cells. For example, CCL2/MCP-1 can increase collagen mRNA levels in cultured fibroblasts [74], and CCR2 and CXCR2 expression has been reported for pulmonary and dermal fibroblasts, respectively [75, 76]. At present it remains unclear if renal fibroblasts express chemokine receptors. So far, immunohistological and in situ hybridization studies of human biopsies have not found expression of CCR2, CCR5 or CXCR4 on intrinsic renal cells, including fibroblasts. Other chemokine receptors have not been tested to date, so the question of the presence of chemokine receptors on renal fibroblasts and their potential function remains open at this point. On the other hand, in vitro studies suggest that activated renal fibroblasts could secrete chemokines like CCL2/MCP-1 and CXCL1/MIP-2 (abstract; Garcia et al, *J Am Soc Nephrol* 12:630A, 2001), which in turn may attract further leukocytes secreting profibrotic cytokines. As renal fibroblasts produce interstitial matrix components including collagen I upon the stimulation by activated macrophages, this indicates that chemokines, either directly or indirectly via macrophage recruitment, contribute to interstitial collagen deposition and fibrosis.

Descriptive studies in animal models of renal disease and human renal biopsies

CCL2/MCP-1 is expressed by tubular epithelial cells in animal models of progressive nephropathies and renal fibrosis [14, 77, 78]. In areas of interstitial fibrosis CCL5/RANTES is predominantly expressed by leukocytes but may be secreted also by interstitial fibroblasts [14, 77]. Consequently, CCR5-positive lymphocytes accumulate in the interstitium and the amount of interstitial CCR5 positive lymphocytes has been found to correlate with serum creatinine levels in human biopsies of various renal disorders [15]. Although diabetic and vascular nephropathies are two major causes of progressive renal disease, only a few studies have been published on the role of chemokines in these disorders. An increased expression of CCL2/MCP-1 was reported in human diabetic nephropathy that correlated with the degree of tubulointerstitial damage and macrophage infiltration [79–81]. In angiotensin II-dependent rat models of hypertensive nephrosclerosis CCL2/MCP-1 expression was increased and was temporally and spatially related to macrophage infiltration [82].

Functional studies in animal models and in patients with renal diseases

The functional role of chemokines in interstitial disease was confirmed by several animal studies using chemokine antagonists or chemokine-deficient mice. Anti-CCL2/MCP-1 antiserum reduced interstitial leukocyte infiltrates and interstitial collagen deposition in mice with nephrotoxic serum nephritis [58]. As mentioned before, CCL2/MCP-1 knockout-mice with nephrotoxic nephritis develop less severe tubulointerstitial damage [59]. We and others have shown that several CC-chemokines and osteopontin are expressed in increasing amounts within the tubulointerstitium after unilateral ureter ligation [14, 83, 84]. We have recently shown that CCR1 blockade substantially reduced interstitial leukocyte infiltration, interstitial fibroblast accumulation, collagen deposition, and interstitial fibrosis after ureter obstruction [85]. The MRL/MpJ Fas$^{lpr/lpr}$ (MRL/*lpr*) mouse, which serves as a model of systemic lupus, develops a chronic progressive immune complex nephritis [77]. In this model inflammation and chemokine expression is initially restricted to the glomerulus. Following the onset of proteinuria mice show increased expression of chemokines in peritubular lesions that correlated with progressive mononuclear cell infiltration and expression of chemokine receptors CCR1, CCR2, and CCR5 [77]. When CCL2/MCP-1–deficient knockout mice were crossbred with MRL/*lpr* mice, CCL2/MCP-1 deficiency reduced proteinuria, renal damage and renal macrophage and T cell recruitment but not proliferation [86]. This suggests that CCL2/MCP-1, presumably via its receptor CCR2, plays an important role in the leukocyte infiltration and resulting tissue injury in the lupus model. Further support of this hypothesis comes from human studies in patients with lupus nephritis where urinary CCL2/MCP-1 levels correlated with the extent of renal disease activity and

macrophage infiltration [87, 88]. Furthermore, glucocorticoid treatment of the lupus nephritis resulted in a prompt reduction in urinary CCL2/MCP-1 levels consistent with in vitro results on the inhibitory effect of glucocorticoids on renal cell production of CCL2/MCP-1 [89].

A link may exist also between angiotensin II and chemokines in renal disease [90]. Blockade of the renin-angiotensin system is currently the mainstay of treatment for progressive renal disease. In this context it is interesting that enalapril and losartan reduced renal chemokine expression in MRL/*lpr* mice in correlation with improved renal function and renal damage (Perez de Lema, manuscript submitted for publication). Renal MCP-1 expression was reduced with angiotensin blockade in the diabetic rat, during rat immune complex glomerulonephritis, the anti-Thy1.1 model, and after unilateral ureter ligation [91–94].

In summary, these findings point toward a role for chemokines and their receptors in leukocyte-mediated progressive tubulointerstitial damage and fibrosis, which ultimately leads to end-stage renal disease. Conversely, blockade of tubulointerstitial leukocyte recruitment and activation by antagonism of chemokine actions may mitigate chronic renal inflammation and subsequent fibrosis, a hypothesis deserving further evaluation.

CHEMOKINE INVOLVEMENT IN ACUTE AND CHRONIC TRANSPLANT REJECTION

Expression of inflammatory chemokines, including CCL5/RANTES, CCL2/MCP-1, CXCL10/IP-10, and XCL1/lymphotactin, increases during acute and chronic allograft rejection [31]. In a rabbit model of acute renal allograft rejection renal function and tubulointerstitial damage improved when CCR1 was blocked with the small peptide antagonist BX471 [95]. Blockade of the chemokine receptor CXCR3 (binding CXCL9/Mig, CXCL10/IP-10, and CXCL11/I-TAC) and the CX_3CL1/fractalkine receptor CX_3CR1 by neutralizing antibodies led to prolonged allograft survival in murine heart transplantation models [96, 97], but their role in renal allograft rejection has not yet been evaluated. In rat models of acute renal allograft rejection treatment with the CCL5/RANTES antagonist Met-RANTES, which blocks the CCL5/RANTES receptors CCR1 and CCR5, significantly reduced the vascular and tubular damage and suppressed mononuclear cell infiltration associated with acute rejection [98]. These results were confirmed and expanded on in a recent study where application of Met-RANTES for seven days after renal transplantation in rats improved chronic transplant function with reduced proteinuria, glomerulosclerosis, and interstitial fibrosis after 28 weeks, supporting a role for chemokine antagonism for prevention of chronic transplant loss [99]. As leukocytes expressing the CCL5/RANTES receptor CCR5 are frequently found in interstitial cell infiltrates of human renal transplants [15], these data may be relevant to human transplant rejection also. The strongest support for this hypothesis comes from a recent multicenter case control study in human renal transplant recipients [100]. In humans a 32 bp deletion (Δ32) in the *CCR5* gene generates a nonfunctional receptor, which in the homozygous state shows a prevalence of about 1% in the Caucasian population. The Δ32 homozygotes show no phenotype and represent essentially a CCR5 "knockout" population. This population was discovered as the lack of CCR5 protects from infection with M-tropic HIV, which uses CCR5 as a coreceptor [101]. As in chronic transplant rejection ligands for CCR5 are up-regulated, and the graft is infiltrated by CCR5-positive mononuclear cells, the influence of the Δ32 mutation of CCR5 was analyzed retrospectively in renal transplants [100]. Out of over 1200 recipients of renal transplants, 21 (1.7%) were identified as homozygous for the CCR5Δ32 mutation. Graft survival was significantly better in the homozygous Δ32CCR5 group compared to either heterozygote or wild-type transplant recipients, suggesting a pathophysiological role for CCR5 in transplant loss [100]. A detailed analysis of various chemokine receptor polymorphisms on the frequency of acute rejection episodes of human renal transplants was reported in a recent study [102]. Because of the smaller number of patients examined no homozygotes for the CCR5Δ32 mutation were present. However, evaluation of another mutation of CCR5 (homozygous for the 59029-A allele) as well as the CCR2 deletion (CCR2-64I allele) significantly reduced the frequency of acute rejection episodes.

Therefore, CCR5-mediated leukocyte influx and activation may be operational not only in acute transplant rejection, but also in chronic transplant survival. Thus, CCR5 may be a reasonable target for therapeutic intervention, especially as the absence of CCR5 in humans is not associated with any deleterious phenotype. Furthermore, CCR5 may be a therapeutic target for non-transplant-related progressive nephropathies

CHEMOKINES CAN BE INVOLVED IN RESOLUTION OR PROGRESSION OF RENAL DISEASE

Persistent exposure to triggers of renal injury is an important factor for disease progression while removing triggers may eventually result in resolution of the inflammatory response with restoration of normal renal architecture and function. Unfortunately, renal damage, once established, has a tendency to progress even if the initial insult has subsided. What then could be the mechanisms that determine either the resolution or the progression of renal disease, and what role might chemokines play in influencing the outcome?

To date the mechanisms that control resolution or persistence of a leukocytic infiltrate in the kidney have not been elucidated. Clearly, the normal glomerular architecture can be completely restored despite marked macrophage infiltration and mesangial hypercellularity during the active phase of immune complex glomerulonephritis [103]. In this context it may be of interest that in a model of transient immune complex glomerulonephritis, CCL2/MCP-1 and CCL5/RANTES were up-regulated in the early initiation phase but were already down-regulated at the phase of maximal proteinuria and glomerular macrophage infiltration [13]. In addition to the transient expression of chemokines by most cell types in vitro, the down-regulation of chemokine synthesis by locally generated factors such as prostaglandins, TGF-β, or induction of leukocyte apoptosis may terminate chemotactic signaling in vivo [103–106]. During acute disease states, termination of the trigger injury therefore correlates with a reduction of chemokine expression by intrinsic renal cells and infiltrating leukocytes. As further influx of leukocytes is impaired, the number of infiltrating leukocytes declines in parallel to restoration of the normal architecture that corresponds to the resolution of glomerulonephritis in post-infectious states [13]. Whether infiltrating leukocytes are mainly removed by apoptosis in situ [107] and or partially emigrate via the lymphatics or venules has not been studied in detail. It is important to note that termination of the chemokine signal is critical for the resolution of the inflammatory process. On the other hand, if local chemokine expression is augmented by another stimulus, a pre-existing renal disease may eventually progress to severe renal damage. This scenario may be pertinent to renin-angiotensin activation secondary to renal disease or to infection triggering exacerbation of renal disease. As noted earlier, inhibition of angiotensin reduces renal chemokine production and leukocyte infiltration in both hemodynamic- and immune-mediated renal disease models [77, 90–94]. This mechanism potentially contributes to the proven therapeutic effects of angiotensin blockade in the progression of renal disease.

Intercurrent infections frequently result in a deterioration of renal diseases including chronic transplant nephropathy. The proinflammatory signals of bacterial and viral invasion are mediated by a group of mammalian receptors, the Toll-like receptors-TLRs [108]. These receptors recognize components of the infectious agents such as endotoxic lipopolysaccharides, peptidoglycanes, and unmethylated DNA [108]. In support of such a mechanism contributing to deterioration of renal disease are data showing endotoxin-induced aggravation of experimental GN [109]. Furthermore, we recently showed that injection of CpG-oligonucleotides mimicking bacterial DNA into mice with otherwise self-limiting apoferritin-induced glomerulonephritis resulted in a severe exacerbation and progression instead of resolution of the disease process. This was associated with increased chemokine and chemokine receptor expression in the diseased kidneys [Anders et al, *J Am Soc Nephrol* 2003 (in press)]. Thus, even if the triggering injury subsides, the renal chemokine expression can be maintained by other mechanisms such as infection, renin-angiotensin activation, hypoxia or proteinuria, and contribute to persistent leukocyte infiltration and tissue damage.

An essential factor in the switch between resolution and progression may be the extent of irreversibly damaged renal architecture, which in turn depends on the pattern of renal injury. Diffuse disease processes have a greater potential to induce progressive renal disease compared to focal renal injury. If the nephron integrity is destroyed, this may result in threatened tissue homeostasis with cellular stress (hypoxic, mechanical, metabolic, nutritional), turning into inflammation with ongoing immune responses such as fibrosis and, thus, resulting in a vicious cycle. In fact, the complicated anatomical and functional structure of the kidney, including its unique vascular network, may predispose this organ to progressive disease.

THERAPEUTIC IMPLICATIONS FOR PROGRESSIVE RENAL DISEASE

Despite the somewhat conflicting experimental results obtained with chemokine antagonists and knockout mice, specific chemokine receptors may be promising therapeutic targets for the use of antagonists in many inflammatory disease processes that involve the infiltration of leukocytes (Fig. 3) [110, 111]. Other families of seven-transmembrane-spanning receptors have proven to be valuable targets for specific therapeutic receptor blockage, for example, the β-adrenergic blocker. For the blockade of the leukocytic cell infiltrate in chronic renal disease, the chemokine receptors CCR1, CCR2, CCR5, CXCR3, and CX3CR1 should be suitable targets [13, 14, 61, 77, 112–114]. However, for antagonism of each of these targets, potential systemic immunomodulatory side effects have to be carefully evaluated. Unfortunately, at present a major problem for testing small molecule antagonists is the species specificity. Most chemokine antagonists have been developed for the human system and unfortunately their antagonistic activity in rodents is mostly minimal, precluding their testing in rodent models and explaining the lack of in vivo results. Some information is available about the blockade of CCR1 during chronic inflammatory disease processes. Tokuda et al have developed a polyclonal antibody with blocking activity against CCR1 [115]. When given in the murine model of bleomycin-induced pulmonary fibrosis, a marked reduction of leukocytic cell infiltrate and interstitial fibrosis was found. We and others have found beneficial effects of CCR1 blockade with BX471, a small molecule antagonist

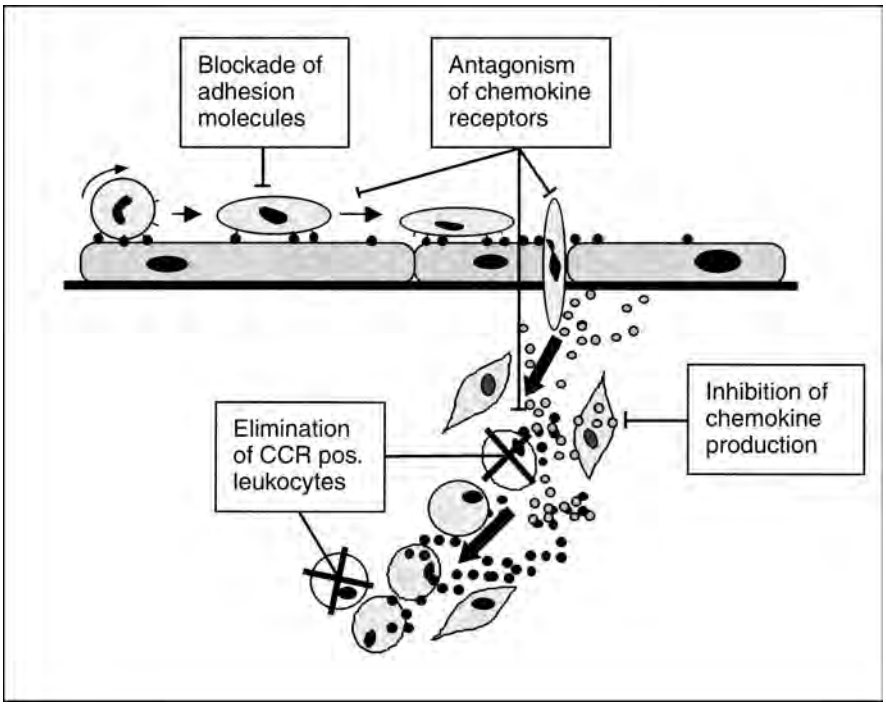

Fig. 3. Mechanisms of leukocyte transmigration and possible sites for therapeutic intervention. Activated endothelium expresses adhesion molecules and presents chemokines on the luminal membrane, which supports selective binding to the corresponding adhesion molecules on circulating leukocytes. Rolling of cells and subsequent firm adhesion is followed by spreading of the cell, which involves activation of integrins by interactions of chemokines and their respective chemokine receptors. Leukocyte transmigration requires temporary disintegration of cell-cell contacts and loosening of the basement membrane, and is followed by leukocyte migration along the chemokine gradient into the area of inflammation and chemokine production. Potential sites for chemokine antagonists as therapeutic principles include: antagonism of chemokine-mediated leukocyte adhesion, activation, *trans*migration, and migration along the chemokine gradient. An alternative strategy would involve elimination of chemokine receptor positive leukocytes by either single chain, bispecific antibodies involving T cell activation or by chemokine-cell toxin constructs.

against human and murine CCR1 on renal fibrosis in the hydronephrotic kidney or in a model of rabbit renal allograft rejection [85, 95]. Studies with other CCR1 antagonists using other immune-mediated disease models (such as, collagen-induced arthritis) support the hypothesis that CCR1 blockade may be an effective approach for the therapy of inflammatory disease states that involve tissue damage by local leukocyte infiltration [115].

An additional therapeutic strategy, especially after the leukocyte infiltration has already occurred, could be the targeted elimination of immune cells identified by a specific chemokine receptor. Thus, depletion of CCR5-positive T cells and monocytes is possible by bispecific single chain antibodies that bind simultaneously to CCR5 on the target cell and CD3 on T cells, thus activating the T cell to eliminate the CCR5-positive leukocytes [117]. Another possibility to eliminate CCR5 positive cells is represented by a fusion protein consisting of the chemokine CCL5/RANTES and a truncated version of *Pseudomonas* exotoxin A, which specifically destroys the target cell after binding to their CCL5/RANTES receptors such as CCR5 [117]. Elimination of leukocyte subsets identified by a particular chemokine receptor within the inflamed tissue may represent a novel therapeutic strategy of selective immunosuppression. This approach would avoid systemic side effects secondary to leukopenia or systemic immunosuppression, but may well turn out to be limited by other unwanted reactions.

In fact, like any effective agent, chemokine antagonists may induce adverse reactions. For example, Met-RANTES and AOP-RANTES, two CCL5/RANTES antagonists, can aggravate glomerular damage and proteinuria in mice with immune complex glomerulonephritis despite a reduction of glomerular leukocyte infiltration (abstract; *J Am Soc Nephrol* 12:651, 2001). The variable effects of AOP-RANTES in different models of glomerular injury may relate to different systemic immune mechanisms in these models [62, 109]. Exacerbation of renal disease by targeting of single chemokine receptors has been found also in knockout mice for the chemokine receptors CCR1 and CCR2, as mentioned earlier in this article [65, 66]. Although effects that occur in chemokine receptor knockout mice may be different from those occurring with short-term use of receptor antagonists, the present data indicate that therapeutic targeting of chemokine receptors, under certain conditions, may exacerbate underlying disease. The exact mechanisms of the apparent modulatory effects of chemokine receptors in acute renal inflammation remain to be elucidated. Although beneficial effects of chemokine blockade at present can be explained best by the prevention of leukocyte infiltration, their effects on the phenotype, survival or immigration of infiltrated cells as well as their effects on the overall immune response including, for example, dendritic cells, memory T cells, B cells and antibody production, and even potential effects on renal parenchymal cells must be considered. Potentially the expression of chemokine receptors on intrinsic renal cells, such as CCR1 and CCR7 on mesangial cells, may contribute to unexpected effects of chemokine receptor knockouts

and chemokine antagonists in experimental renal diseases [52, 53]. As chemokine receptors are involved in many other physiological processes, potential side effects of receptor blockade must be carefully evaluated before approaching treatment studies in human disease.

SUMMARY

Chemokines and chemokine receptors are involved at multiple stages of the inflammatory response in renal disease including the multistep process of leukocyte adhesion, transmigration, and differentiation into the tissue specific phenotype. Multiple strategies for the blockade of chemokine receptors or the specific elimination of chemokine receptor bearing infiltrating cells are being developed. Studies in models of progressive renal disease already illustrate the potential of chemokine antagonists to reduce interstitial leukocyte and fibroblast accumulation, renal fibrosis, and tubulointerstitial damage. On the other hand, potential problems related to unforeseen immune modulation by chemokine antagonists must be kept in mind. Nonetheless, in view of the present data, chemokine receptor antagonists appear to have a great potential as new therapeutic tools in the treatment of progressive renal failure.

NOTE ADDED IN PROOF

Recently, the infiltration of bone marrow–derived cells into interstitial fibrotic lesions and their contribution to interstitial collagen expression has been demonstrated in mice after unilateral ureter ligation (Iwano N, Plieth D, Danff TM, *et al*: Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* 110:341–350, 2002).

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